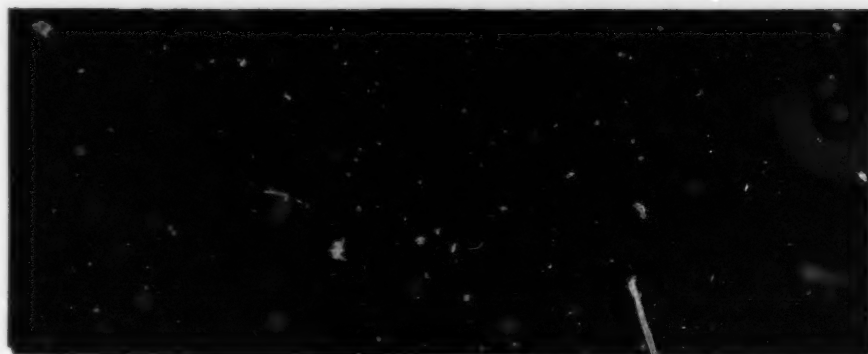




Saskatchewan
Agriculture

Agriculture Development Fund





91-02589/

Technical/Final Report
to
Saskatchewan Agriculture Development Fund

Development of a Vaccination Technique
Against Somatostatin to Improve Growth

R-86-01-0062

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FINAL
TECHNICAL/~~PROGRESS~~ REPORT

DEVELOPMENT OF A VACCINATION TECHNIQUE AGAINST SOMATOSTATIN TO IMPROVE GROWTH.

BACKGROUND AND OBJECTIVES

Somatostatin is a peptide hormone consisting of 14 amino acids. It is secreted from the hypothalamus and has been shown to strongly inhibit growth hormone secretion from the pituitary gland. It is desirable to increase the rate of secretion of growth hormone from the pituitary gland because higher levels of growth hormone in the blood result in higher average daily gains, leaner carcasses, better feed efficiency and increased milk production.

The objective of our on-going research is to develop a method which will neutralize somatostatin. This can be achieved by immunizing the animal against its own circulating somatostatin. The antibodies thus formed will neutralize the endogenous somatostatin, leading to increased levels of growth hormone in the blood.

There are several aspects to the development of the somatostatin vaccine which need to be resolved:

- 1) Choice of carrier protein? Somatostatin itself is not immunogenic. By coupling somatostatin to a foreign protein, the entire conjugate becomes immunogenic. It is not clear which carrier protein (ovalbumin, KLH, hemocyanin) is best.
- 2) Choice of chemical coupling method? The manner in which somatostatin is coupled to the carrier protein is important. There is a high probability that in many instances somatostatin is not coupled properly in that the epitope, the biologically important region, is not displayed. The efficiency of coupling can be influenced by the choice of chemical reagents and reaction conditions.
- 3) Frequency of the somatostatin in the somatostatin-carrier protein conjugate is important. Ideally a frequency of 5 to 15 moles of somatostatin per mole of carrier protein is considered ideal for an optimal immune response.
- 4) Choice of adjuvant? The somatostatin vaccine by itself is still weakly immunogenic and a strong immune response is required. Therefore adjuvants are included in the vaccine to help mount a vigorous immune response. Unfortunately, there is little information available as to which adjuvant is most appropriate. Therefore, the somatostatin vaccine must be evaluated in conjunction with a variety of different adjuvants.
- 5) Dosage of the vaccine and frequency of vaccination in terms of generating a quick and vigorous immune response?
- 6) Level of immune response required to achieve improvement in animal performance?

7) Affinity of the antibodies produced towards somatostatin? The receptor for somatostatin on the pituitary gland has a high affinity for the hormone. Therefore, in order for the antibodies to neutralize the somatostatin, they must out compete the receptor. The antibodies generated in the immune response must not only be high in number, but also high in affinity.

EXPERIMENTAL METHODS, RESULTS AND DISCUSSION

EXPERIMENT SRIF-3

This experiment was designed to evaluate the method of conjugation. Two reagents were used: carbodiimide (EDC), which is a non-selective coupler and N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) which couples the α -NH₂ group with NH₂ groups on the carrier protein through formation of a disulfide bond. Both reagents were used as recommended in the literature. Somatostatin (SS) was coupled to ovalbumin.

Lambs (22 kg) were assigned to three treatments (n=15): control, EDC vaccine, SPDP vaccine. The control treatment consisted of ovalbumin (1 mg), the EDC vaccine contained 250 ug SS equivalent and the SPDP vaccine 50 ug SS equivalent. Freund's Complete Adjuvant was used. Lambs were injected four times at two week intervals. Blood was routinely sampled and weight gains were recorded.

Lambs immunized with both SPDP and EDC vaccines slowly (after 6 weeks) developed a titer of 1:100. There was no difference between the two treatments, even though SPDP contained only 1/5 of the SS conjugate. No difference in overall gain was observed, but the EDC treatment did result in a transient growth acceleration. Affinity of the antibodies for the EDC treatment was low, resulting in a lack of neutralizing ability. The yield of the SPDP conjugate was low and the method of preparation cumbersome. The Alberta Peptide Institute, Edmonton is currently preparing conjugates by solid phase synthesis. These preparations will be tested in lambs, pigs and cattle.

EXPERIMENT SRIF-5

This experiment was also designed to test the coupling of SS, but to a defined carrier: a carbohydrate polymer. AH-Sepharose displays a large number of carboxyl groups. With the use of EDC coupling of SS is more controlled since no NH₂ groups are available on the carrier. The resulting SS-Sepharose conjugate displays a high frequency of SS. Seventy lambs were assigned to seven treatments (n=10), consisting of a control, two vaccine treatments, SS-Sepharose and classical SS-alpha globulin, in combination with three adjuvants: Freund's Complete Adjuvant (FCA), Havlogen and Dextran. Lambs were injected four times (100 ug SS equivalent) at two-week intervals. Blood samples were taken and growth performance recorded.

The Havlogen and Dextran adjuvants were both ineffective in inducing an immune response. FCA was partially effective in that 2 lambs from the SS-Sepharose and 4 lambs from the SS-alpha globulin treatments responded. The poor response may be attributed to the relatively low dosage of the SS. No

difference in growth was recorded.

EXPERIMENTS SRIF 6, 7, 8

The objective of these experiments was to evaluate various commercially available adjuvants for use in somatostatin-immunization in pigs, lambs and rats. One common conjugate was prepared for all species by coupling somatostatin to ovalbumin. Pigs and lambs were injected with 250 ug somatostatin equivalent and rats with 10 ug equivalent.

Treatments: Rats (n=15): Adjuvants Havlogen, Havlogen with Regressin
Pigs (n=4): Adjuvants FCA, Havlogen, Ribit, Regressin
Lambs (n=15): Adjuvants Havlogen, Ribit, Havlogen with Regressin

All animals were injected twice, two weeks apart. Blood samples were taken 24 and 50 days after injection.

All adjuvants except FCA failed to induce a vigorous immune response. However FCA can not be used since it renders animals positive to the tuberculin test. The low immune responses observed may be related to a lack of depot effect (slow release). Recent studies have shown that very frequent vaccination with these adjuvants is effective but this lacks appeal in terms of applied livestock production. We intend to evaluate other adjuvants and delivery systems such as liposomes.

EXPERIMENT SRIF-10

Evaluation of somatostatin-KLH and somatostatin-ovalbumin conjugates produced by solid phase peptide synthesis and conjugation of the protected peptide with ovalbumin by photolysis with subsequent deprotection. This technique allows a very precise control of conjugation, allowing for a high frequency of somatostatin coupling to the carrier, with appropriate projection of the epitope. The Alberta Peptide Institute prepared conjugates with two different carrier proteins: KLH and ovalbumin. Initially these two conjugates will be tested in rats.

Treatments (n=15): SS-KLH, SS-ovalbumin and control with use of FCA as adjuvant. Dosage of 10 ug SS equivalent, twice, 14 days apart. Rate of gain and antibody titers will be monitored.

The SS-KLH conjugate was very poorly soluble, probably due to many of the polar groups on KLH being used for SS coupling sites. Rats immunized with SS-KLH did not develop antibody titer and did not show a change in rate of gain. The SS-ovalbumin conjugate was very soluble. Rats immunized with this conjugate showed a 9% improvement in average daily gain over the seven-day period after the first immunization. This study is still in progress but the initial result is encouraging. Antibody titers, as yet, have not been determined.

EXPERIMENT SRIF-12

We observed that ewe lambs used in experiments SRIF-3 and 5 and retained

for breeding, had developed very high antibody titers against somatostatin. In fact, titers at the termination of studies SRIF-3 and 5 often were in the range of 1:100, whereas 6-9 months later the titers were from 1:5000 to 1:10,000. It appears therefore that after the initial immunizations the ewe lambs may have become autoimmune against somatostatin. Thus the animal's own somatostatin kept triggering and enhancing the immune response. In addition we observed that the antibodies were of very high affinity, approximately one or two magnitudes higher than the affinity of the pituitary somatostatin receptor.

During the first lambing we observed that these ewe lambs had a much higher lambing percentage than control ewe lambs (204% vs 160%). This suggests that somatostatin immunization also could affect fertility. There is some evidence in the literature which indicates that high somatomedin and growth hormone levels in blood are associated with improved fertility.

These immunized ewes have just completed their second lambing. In this particular study, since the ewes have high antibody titers, the emphasis is on animal performance and hormonal changes both in the lambs and ewes. We presently have 13 lambs which acquired a large amount of somatostatin-antibodies through colostrum. Their antibody titers range from 1:3000 to 1:7000. Performance and hormonal changes are compared to lambs from control ewes. At the age of three weeks the lambs will be catheterized and pituitary and thyroid function tests will be performed.

After weaning feed intake, feed efficiency and slaughter quality will be assessed. The nature of the antibodies in lambs will be assessed as well as affinity. While the data are very preliminary (February 19, 1987) we did observe that there is considerable transfer of antibodies against somatostatin in colostrum. The lambs had very high antibody levels, in fact probably sufficiently high to maintain high antibody levels up to slaughter. Also, we observed that these immunized lambs had a significantly higher rate of gain (19% higher than controls, $P < 0.05$).

CONCLUSIONS AND IMPACT

A) Induction of immunity against somatostatin is difficult due to lack of a suitable commercial adjuvant. Anti-somatostatin antibody levels rise slowly and titers are too low to expect improvements in animal productivity. Satisfactory titers were achieved in pigs within three weeks of first immunization using Freund's Complete Adjuvant, which clearly indicates that adjuvanticity of other adjuvants is limited. We will evaluate additional adjuvants, notably liposomes, for their adjuvant properties in somatostatin immunization. Since liposomes are not commercially available, they must be prepared in the laboratory. Choice of carrier protein (ovalbumin) and method of coupling (solid phase peptide synthesis and conjugation) appear to have been resolved.

B) There is some concern that the time required to reach satisfactory antibody levels through active immunization may be too long (4-8 weeks) for swine and lambs destined for slaughter. Thus the time needed for proper immunization in proportion to the total life time of a pig or lamb is relatively long. A

growth response may not be observed until adequate antibody levels have been obtained.

C) We have observed that lambs immunized in several experiments became autoimmune to somatostatin. Interestingly, the antibody levels achieved initially were not very high. To our surprise, antibody levels 6-9 months later were very high. This suggests that the immune system was continuously stimulated by the animal's own natural somatostatin. In addition the antibodies were of high affinity. This finding is important in that animals eventually will develop high antibody levels without the need for repeated vaccinations. This has important implications for cattle production, where the time available to induce an adequate immune response is much longer. In addition, this is important in relation to generating permanent immunity in sows, ewes and cows for the purpose of transferring anti-somatostatin antibodies through colostrum to the newborn.

D) We have some limited evidence which suggests that immunization of ewes against somatostatin resulted in an increased lambing percentage. We intend to explore this finding further in sows and cows.

E) From an experiment presently in progress we have evidence which strongly suggests that transfer of passive immunity through colostrum of immunized dams to newborn may be an attractive method of growth promotion. The newborn immediately receive very large amounts of antibodies resulting in very high antibody titers. The growth promoting effect appears to be evident from birth. It also appears that the levels of antibodies may be sufficiently high to last until slaughter.

Passive immunization of piglets and lambs is attractive for the following reasons:

- 1) Far fewer vaccinations are required. Only the dam requires booster vaccinations. Removing the need for vaccination of piglets reduces the total number of vaccinations per sow and piglets/year fifteen-fold, which is a large economic saving to the producer.
- 2) Growth performance in lambs and piglets appears to be evident immediately at birth.
- 3) The method removes the need for the use of direct growth promoting techniques in the lamb or piglet itself. This may appeal to the consumer.
- 4) Since the sow or ewe have permanent immunity and perhaps autoimmunity high antibody levels would be present. This could have an additional beneficial impact: a) There is evidence suggesting that milk production may be improved, resulting in improved nutrition of piglets, lambs and calves; b) It may improve fertility resulting in a larger number of piglets/sow or lambs/ewe and in better conception rates in cattle.

PAPERS AND PRESENTATIONS:

FORMAL:

- B. Laarveld, R.K. Chaplin and D.E. Kerr, 1986. Somatostatin immunization and growth of lambs. Canadian Journal of Animal Science 66:77-83.
- B. Laarveld, 1986. Potential of immunization against somatostatin as a method of growth promotion. Eli-Lilly Research Laboratories, Greenfield, Indiana; June 3.
- B. Laarveld, 1986. Modulation of growth in ruminants through hormonal manipulation. Nutrition Day Conference, Macdonald College of McGill University, Ste. Anne de Bellevue, Que; April 2.
- B. Laarveld, 1987. Growth hormones. Saskatchewan Provincial Dairy Convention, Regina Inn, Regina; February 11.
- B. Laarveld, 1986. Saskatchewan DHAS supervisor meeting, Circle 8 Building, Saskatoon. Somatotropin in dairy production; 90 minute lecture; October 2.

RADIO OR TELEVISION:

September 8, 1986, CJWW radio; Growth hormones.

February 10, 1987, CJWW radio; Somatotropin for dairy cows.

March 2, 1987, CBC television 'Countryside'; Somatotropin and stimulation of milk production in dairy cows.

INFORMAL PRESENTATIONS:

Several presentations to R.O.P. Committees, SDA extension specialists, Chinese Trade Delegations (2) on new techniques of promoting growth in farm animals and new methods of selecting genetically superior animals.

PERSONNEL INVOLVED IN THE PROJECT:

Mrs. Hampton, Research Technician	80%
Mr. Korchinski, Animal Technician	80%
Ms. J. Hamilton, Research Assistant	5%
Mr. A. van Kessel, M.Sc. student	100%
Dr. P.A. Thacker, Assistant Professor	5%
Dr. R.K. Chaplin, Associate Professor	10%
Dr. B. Laarveld, Associate Professor	30%

ABSTRACT

Immunization of farm animals against somatostatin has potential as a method for promoting growth and lactation. Somatostatin is a hormone which strongly inhibits secretion from the pituitary gland. Thus, removal of somatostatin through immunization results in higher blood growth hormone levels. A series of studies determined that the immune response to the somatostatin-ovalbumin vaccine is weak. Commercially available adjuvants such as Alhydrogel, Havlogen, Ribi, Regressin do not have sufficient adjuvanticity to induce a strong immune response. Studies are in progress to identify or develop an adjuvant which will serve both as a carrier and as an immunogenic stimulator.

SUMMARY

Somatostatin is a peptide hormone consisting of 14 amino acids. It is secreted from the hypothalamus and has been shown to strongly inhibit growth hormone secretion from the pituitary gland. It is desirable to increase the rate of secretion of growth hormone from the pituitary gland because higher levels of growth hormone in the blood result in higher average daily gains, leaner carcasses, better feed efficiency and increased milk production.

The objective of our on-going research is to develop a method which will neutralize somatostatin. This can be achieved by immunizing the animal against its own circulating somatostatin, leading to increased levels of growth hormone in the blood.

A major limitation to the development of the technique is that animals do not produce antibodies against their own hormones. Therefore somatostatin is chemically coupled to a foreign protein such as ovalbumin or egg white. Upon immunization the animal then will mount an immune response to both ovalbumin and somatostatin. The appropriate procedure for coupling the peptide is under development. A second limitation concerns the magnitude of the immune response required. For prevention of disease only low levels of antibodies after vaccination are needed. However for neutralization of somatostatin considerably larger amounts of antibodies are necessary. The immune response normally is stimulated by including an adjuvant in the vaccine. We have evaluated various commercially available adjuvants in lambs, pigs and rats and conclude that none are satisfactory for immunization against somatostatin. We are continuing our studies in this regard. Development of a method of immunization against somatostatin is desirable. Somatostatin immunization has application in all species of farm animals and provides a cheap, safe method of promotion of growth and lactation.

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Project # R-86-01-0062

"Development of a Vaccination Technique Against Somatostatin"

Capital Equipment Inventory

Part of Automatic pipetting station (split between several department research accounts.

Half of lease for 86/87 charges on gamma counter

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"Development of a Vaccination Technique Against Somatostatin"

Personnel Distribution List

J. Hamilton	Student Assistant	100%
C. Hampton	Technician	100%
E. Johnston	Stenographer	10%
N. Webb	Technician	25%

Agricultural Development Fund

Project #R-86-01-0062

"Development of a Vaccination Technique Against Somatostatin"

Financial Projection to March 31/87

Salaries and benefits	\$6,200
Materials and Supplies m	17,330
Equipment	4,220